Supplemental Information

Cross-talk of Cannabinoid and Endocannabinoid Metabolism is mediated via Human Cardiac CYP2J2

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METHODS

Materials. Human CYP2J2 cDNA was obtained from OriGene (Catalog No. SC321730) and modified as published before.[1] Ampicillin, arabinose, chloramphenicol, isopropyl β-D-1-thiogalactopyranoside (IPTG), and Ni-NTA resin were obtained from Gold Biotechnology. δ-aminolevulinic acid was obtained from Frontier Scientific. NADPH was obtained from P212121.com. 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-L serine (POPS) were purchased from Avanti Polar Lipids, Inc. AEA and EET-EAs were obtained from Cayman Chemical. pCBs were purchased as DEA-exempt preparations of each pCB dissolved in methanol to 1.0 mg/mL from Cayman Chemical. All other materials and reagents used were purchased from Sigma-Aldrich and Fisher Scientific.

Expression and purification of recombinant CYP2J2 in *E. coli.* Recombinant D34-CYP2J2 containing a His_5 tag was expressed and purified as previously performed.[1, 2] The D34-CYP2J2 is a 34-residue N-terminal truncation (residues 3-37) of CYP2J2 with a substitution of Leu2 for an Ala residue. These modifications have been previously shown to increase protein yield without affecting activity. [1, 2]

Expression and purification of cytochrome P450 reductase. Expression of cytochrome P450 reductase (CPR) was performed as described previously.[1]

Incorporation of CYP2J2 into Nanodiscs. Nanodiscs (NDs) containing CYP2J2 were prepared in 20% POPS discs as previously described.[3] NDs were used for all experiments except for pCB metabolism experiments.

Soret titration binding. Soret titration binding experiments were performed as previously described.[3] The pCBs were dried with N_2 gas and re-dissolved in DMSO. CBD, CBG, and CBN

produced a Soret shift upon binding and their direct binding was measured. AEA, Δ9-THC, Δ8-THC, and CBC did not produce a significant Soret shift, and so an ebastine (EBS) competitive binding was utilized to measure the binding as previously described for AA.[3]

REFERENCES

[1] McDougle, D. R., Palaria, A., Magnetta, E., Meling, D. D., and Das, A. (2013) Functional studies of N-terminally modified CYP2J2 epoxygenase in model lipid bilayers, *Protein Sci 22*, 964-979.

[2] Zelasko, S., Palaria, A., and Das, A. (2013) Optimizations to achieve high-level expression of cytochrome P450 proteins using Escherichia coli expression systems, *Protein Expres Purif 92*, 77-87.

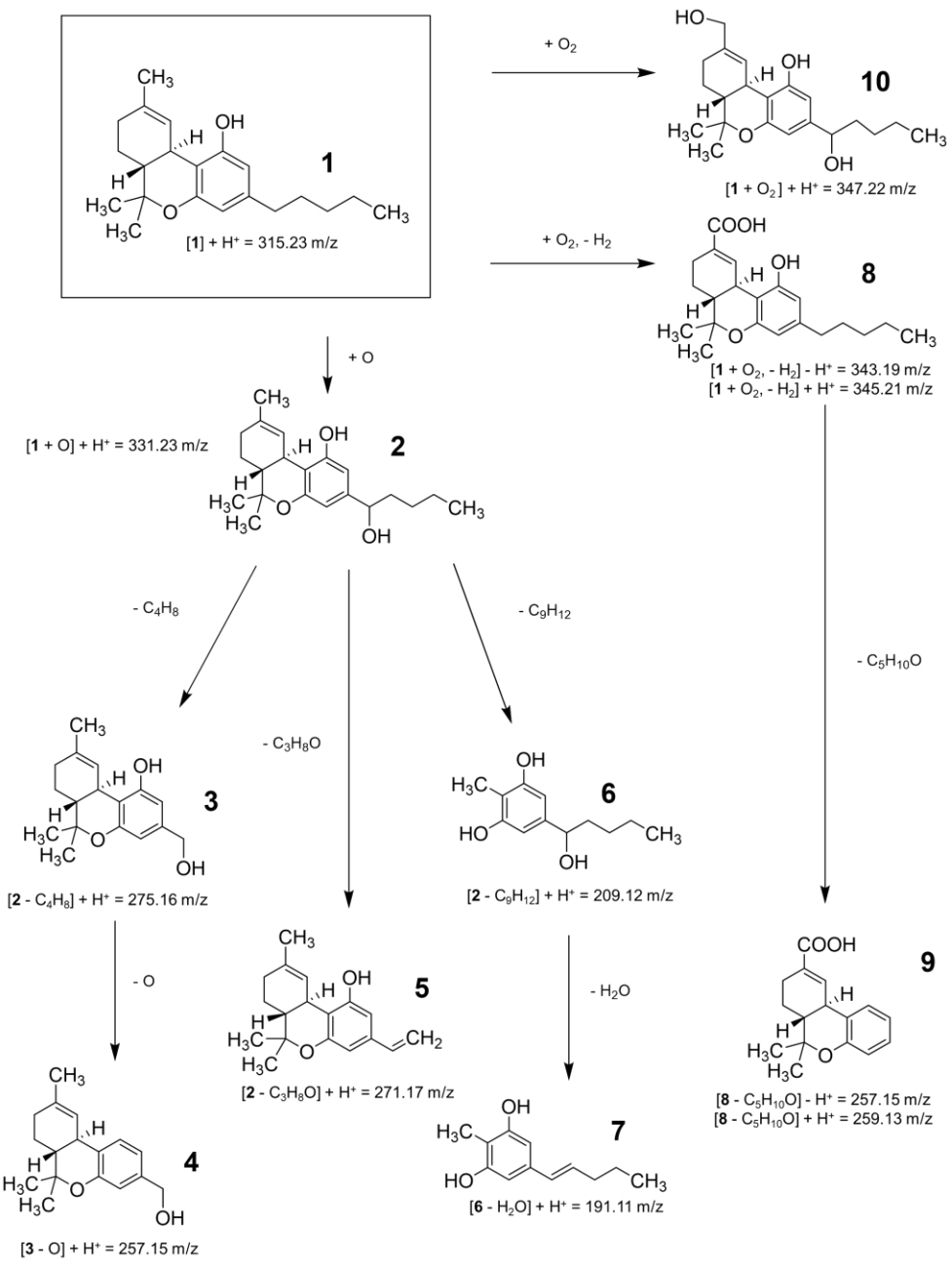
[3] Arnold, W. R., Baylon, J. L., Tajkhorshid, E., and Das, A. (2016) Asymmetric Binding and Metabolism of Polyunsaturated Fatty Acids (PUFAs) by CYP2J2 Epoxygenase, *Biochemistry 55*, 6969-6980.

Guide for reading Supplemental Tables

In each of the Supplemental Tables, **Ions** are classified by the chemical species from which they were fragmented. The unmetabolized phytocannabinoid is referred to as **Parent**, while each respective metabolite is named after its **Modification** (e.g. **1'/1''-OH** for monohydroxylation observed along the pentyl chain). **DiOH** is listed as is, for the exact position for second hydroxylation performed by CYP2J2 could not be determined from the spectral data for every phytocannabinoid tested. In most dehydroxylated species, the 1'/1''-OH is suggested.

Within each listed **Modification**, the number of **Ions**, representing fragments of the chemical species, are listed by row. Relative abundance is given for each species and is calculated as a percentage of the highest intensity **Ion** signal, given the same retention time for the **Ions** considered. Relative abundance values are calculated from MS/MS data unless indicated otherwise.

For example, in **Supplemental Table 1**, **Ion 2**, representing a fragment 1'-OH-Δ9-THC, elutes at 20.42 and 21.05 minutes. The intensities for each of these reported peaks are 3504953.25 and 556748.44, respectively. When calculating each peak's relative abundance, compared to other fragments eluting at the same time, one would compare these intensities to those of the most abundant **Ion**, which would be **Ion 4**, for both retention times. Dividing the reported intensities for **Ion 2** by that of **Ion 4**, at each respective retention time, yields the displayed relative abundance.

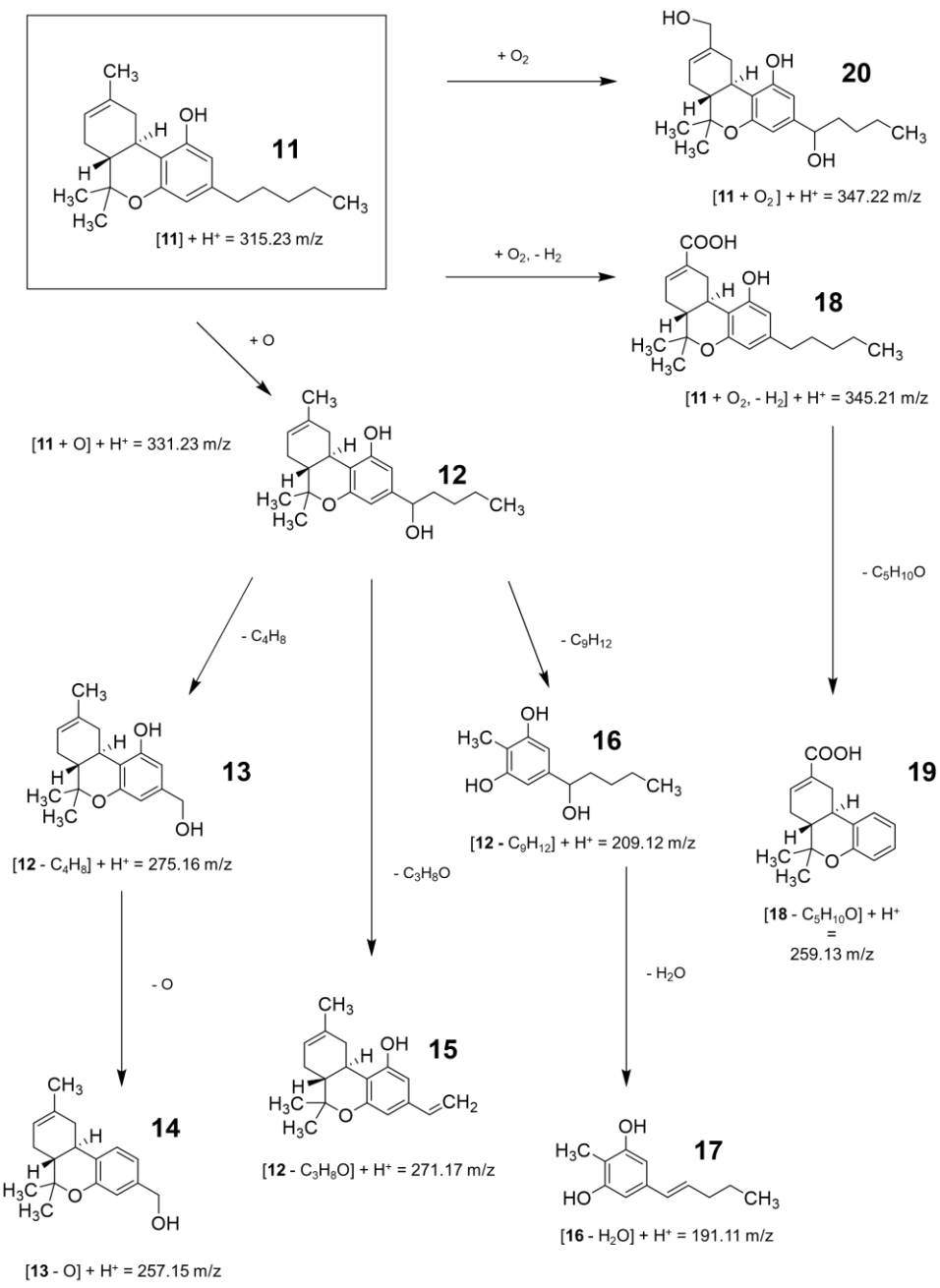


Supplemental Figure 1. Fragmentation pathway of Δ9-THC. Parent species as Ion **1**; monohydroxylated species represented as Ions **2-7**; carboxylated species as Ions **8** and **9**; dihydroxylated species as Ion **10**. Corresponding MS/MS data and retention times provided in **Supplemental Table 1**.

Supplemental Table 1. MS/MS data corresponding to ions distinguishing CYP2J2-Δ9-THC metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Delta-9-tetrahydrocannabinol	**Parent***	**1**	$C_{21}H_{30}O_2$	+	24.09	315.23	75162304.00	100.00
	1'-OH	**2**	$C_{21}H_{30}O_3$	+	20.42	331.23	3504953.25	7.64
				+	21.05	331.22	556748.44	2.51
		3	$C_{17}H_{22}O_3$	+	20.42	275.16	3191566.25	6.96
				+	21.05	275.16	103704.74	0.47
		4	$C_{17}H_{22}O_2$	+	20.42	257.15	45883472.00	100.00
				+	21.05	257.15	22224676.00	100.00
		5	$C_{18}H_{22}O_2$	+	20.42	271.17	1603232.25	3.49
				+	21.05	271.17	302656.03	1.36
		6	$C_{12}H_{18}O_3$	+	20.42	209.12	4297215.00	9.37
				+	21.05	209.12	113166.77	0.51
		7	$C_{12}H_{16}O_2$	+	20.42	191.11	24560034.00	53.53
				+	21.05	191.11	6284094.00	28.28
	11-COOH	**8**	$C_{21}H_{28}O_4$	+	18.76	345.20	29737.60	4.88
				-	18.89	343.19	1077148.75	100.00
				+	19.4	345.21	169284.34	70.20
				-	22.67	343.19	506196.88	58.80
		9	$C_{16}H_{18}O_3$	+	18.76	259.13	23468.31	3.85
				-	18.89	257.15	202751.48	18.82
				+	19.4	259.13	6911.84	2.87
	DiOH	**10**	$C_{21}H_{30}O_4$	+	20.71	347.22	52726.88	6.51

*Parent Ion data gathered from MS/MS data of Δ9-THC standard.



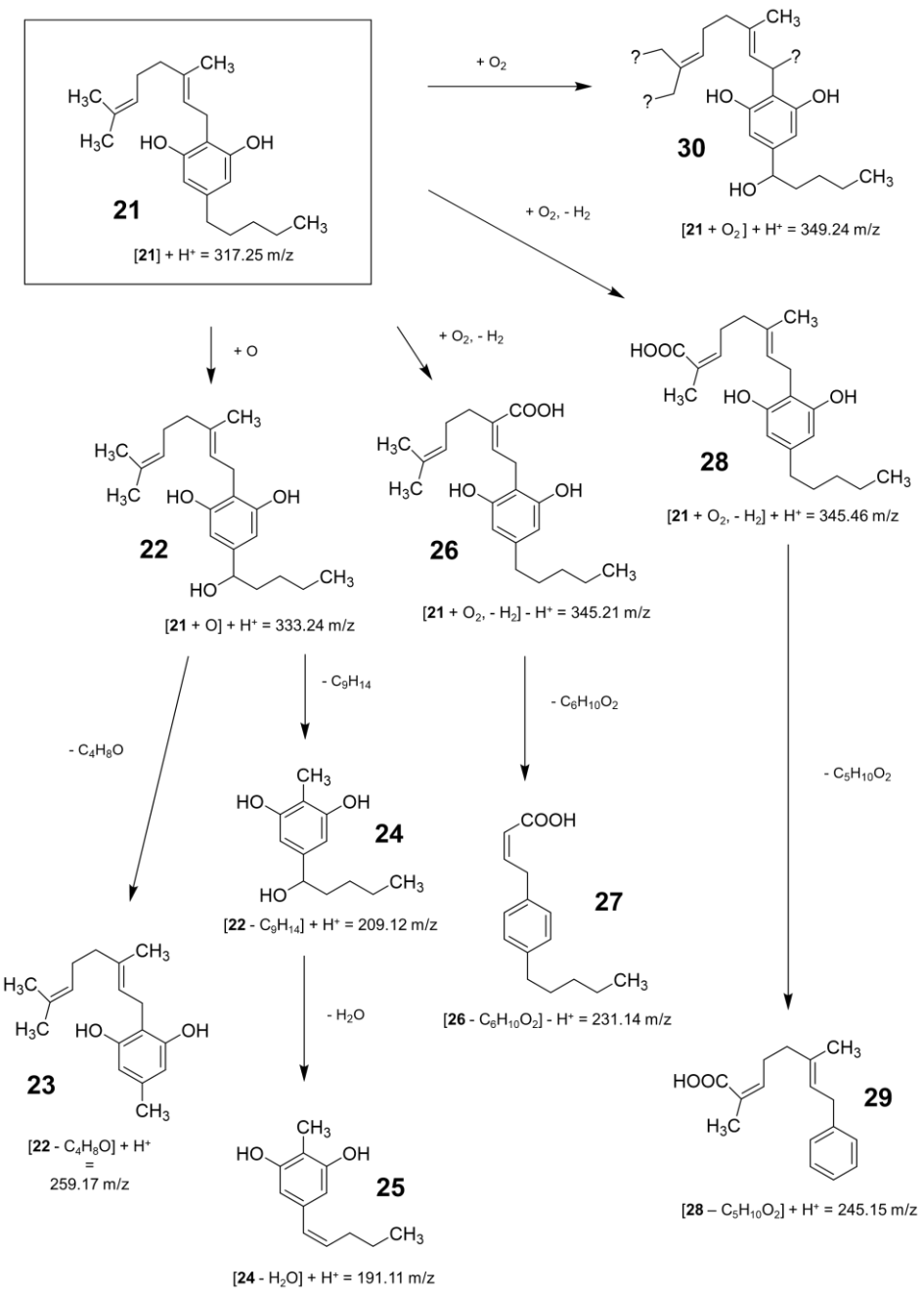
Supplemental Figure 2. Fragmentation pathway of Δ8-THC. Parent species as Ion **11**; monohydroxylated species represented as Ions 1**2-17**; carboxylated species as Ions **18** and 1**9**; dihydroxylated species as Ion **20**. Corresponding MS/MS data and retention times provided in **Supplemental Table 2**.

Supplemental Table 2. MS/MS data corresponding to ions distinguishing CYP2J2-Δ8-THC metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Delta-8-tetrahydrocannabinol	Parent*	11	$C_{21}H_{30}O_2$	+	24.16	315.23	63499332.00	100.00
	1'-OH	12	$C_{21}H_{30}O_3$	+	20.84	331.23	6163016.00	7.20
				+	21.4	331.23	714990.75	1.70
		13	$C_{17}H_{22}O_3$	+	20.84	275.16	16755311.00	19.58
				+	21.4	275.16	181899.47	0.43
		14	$C_{17}H_{22}O_2$	+	20.84	257.15	85588832.00	100.00
				+	21.4	257.15	42145924.00	100.00
		15	$C_{18}H_{22}O_2$	+	20.84	271.17	1189927.88	1.39
				+	21.4	271.17	329652.75	0.78
		16	$C_{12}H_{18}O_3$	+	20.84	209.12	24819734.00	29.00
				+	21.4	209.12	330217.00	0.78
		17	$C_{12}H_{16}O_2$	+	20.84	191.11	42031644.00	49.11
				+	21.4	191.11	13706612.00	32.52
	11-COOH**	18	$C_{21}H_{28}O_4$	+	19.17	345.21	43102268.00	100.00
		19	$C_{16}H_{18}O_3$	+	19.17	259.13	94450.52	21.91
	DiOH	20	$C_{21}H_{30}O_4$	+	16.4	347.22	756208.25	17.06

*Parent Ion data gathered from MS/MS data of Δ8-THC standard.

**Δ8-THC-11-COOH data gathered from MS data, as MS/MS spectra did not provide any ions with a mass corresponding to the unfragmented 11-carboxylated fragment.

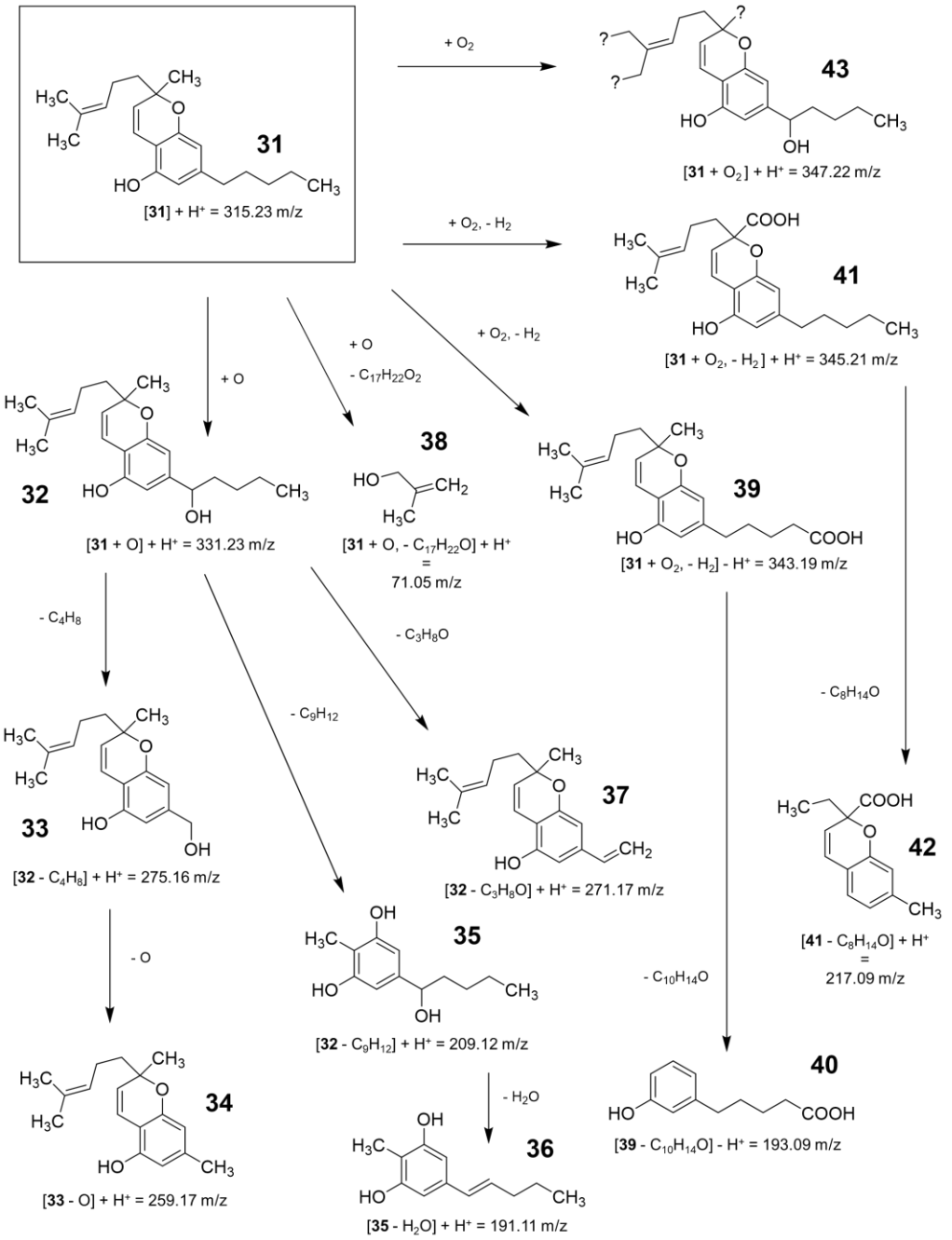


Supplemental Figure 3. Fragmentation pathway of CBG. Parent species as Ion **21**; monohydroxylated species represented as Ions **22-25**; 9'-carboxylated species as Ions **26** and **27**; 5"-carboxylated species as Ions **28** and **29**; dihydroxylated species as Ion **30**, where question marks represent potential positions for subsequent hydroxylation, in addition to the 1" position. Corresponding MS/MS data and retention times provided in **Supplemental Table 3**.

Supplemental Table 3. MS/MS data corresponding to ions distinguishing CYP2J2-CBG metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Cannabigerol	**Parent***	**21**	$C_{21}H_{32}O_2$	+	20.86	317.25	15646022.00	15.04
	1"-OH	**22**	$C_{21}H_{32}O_3$	+	19.03	333.24	80101.83	0.88
		23	$C_{17}H_{24}O_2$	+	19.03	259.17	361966.63	3.99
		24	$C_{12}H_{18}O_3$	+	19.03	209.12	9062598.00	100.00
		25	$C_{12}H_{16}O_2$	+	19.03	191.11	6411131.50	70.74
	9'-COOH	**26**	$C_{21}H_{30}O_4$	-	14.35	345.21	363732.16	8.56
				-	17.96	345.21	209334.03	5.63
				-	22.32	345.21	1130435.75	100.00
		27	$C_{15}H_{20}O_2$	-	17.96	231.14	10928.57	0.29
	8'/10'-COOH	**28**	$C_{21}H_{30}O_4$	+	18.09	345.46	6156.17	0.09
		29	$C_{16}H_{20}O_2$	+	18.09	245.15	9129.97	0.13
	DiOH	**30**	$C_{21}H_{32}O_4$	+	15.07	349.24	16091.57	0.96
				+	15.26	349.20	11280.58	0.85
				+	18.33	349.24	4345.53	0.20

*Parent Ion data gathered from MS/MS data of CBG standard.

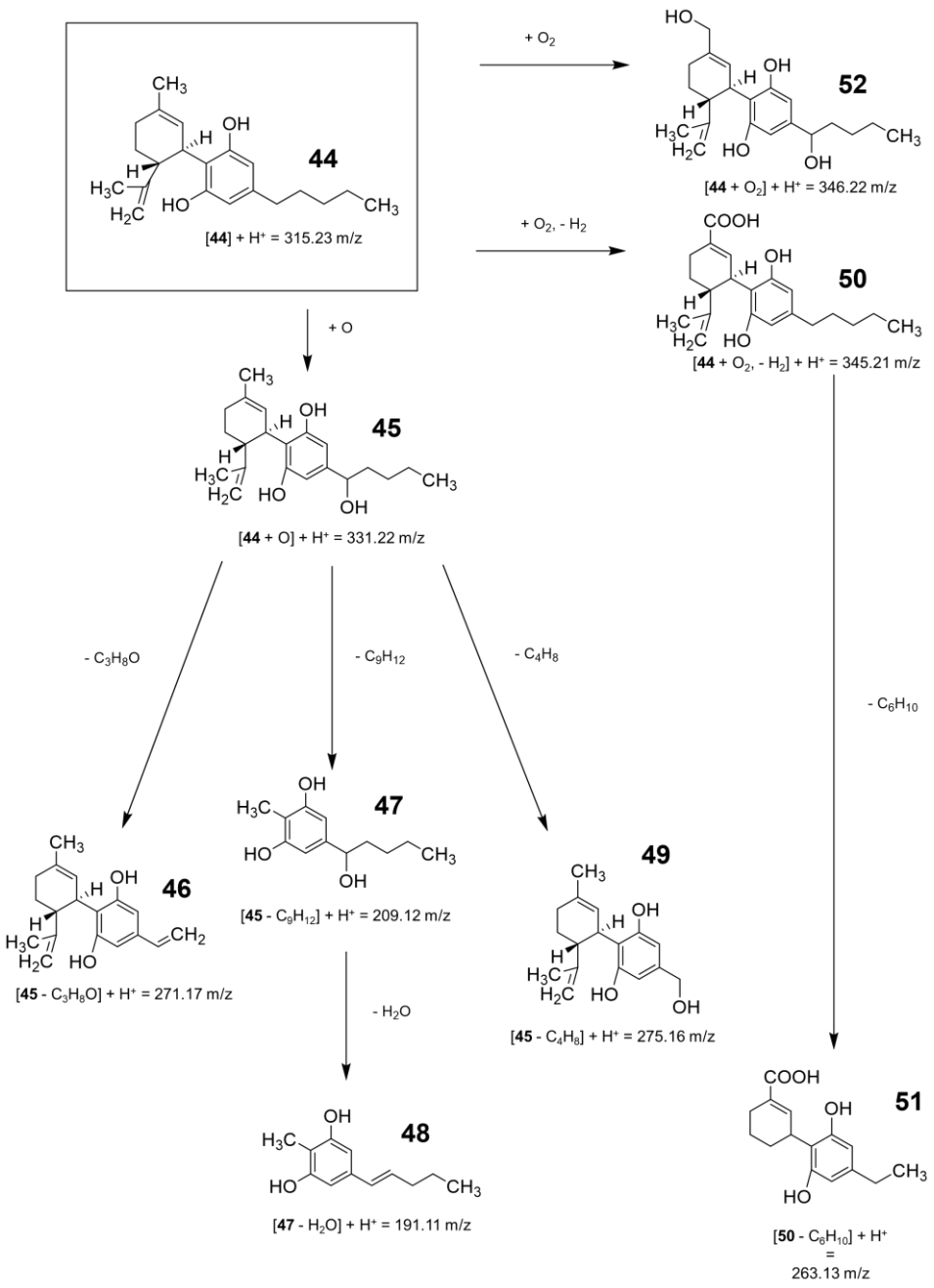


Supplemental Figure 4. Fragmentation pathway of CBC. Parent species as Ion **31**; 1”-monohydroxylated species represented as Ions **32-37**; 8’/10’-monohydroxylated species represented as Ion **38**; 9’-carboxylated species as Ions **41** and **42**; 5”-carboxylated species as Ions **39** and **40**; dihydroxylated species as Ion **43**, where question marks represent potential positions for hydroxylation other than at the 1” position. Corresponding MS/MS data and retention times provided in **Supplemental Table 4**.

Supplemental Table 4. MS/MS data corresponding to ions distinguishing CYP2J2-CBC metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Cannabichromene	**Parent***	**31**	$C_{21}H_{30}O_2$	+	24.98	315.23	99115136.00	100.00
	1"-OH	**32**	$C_{21}H_{30}O_3$	+	21.95	331.23	2570219.00	28.33
				+	25.91	331.23	12169246.00	100.00
		33	$C_{17}H_{22}O_3$	+	21.95	275.16	3260814.25	35.94
		34	$C_{17}H_{22}O_2$	+	25.91	259.17	413954.13	3.40
		35	$C_{12}H_{18}O_3$	+	21.95	209.12	6257679.50	68.98
		36	$C_{12}H_{16}O_2$	+	21.95	191.11	7630927.00	84.11
		37	$C_{18}H_{22}O_2$	+	21.95	271.17	314709.53	3.47
				+	25.91	271.17	1348963.75	11.09
	8'/10'-OH	**38**	C_4H_8O	+	25.91	71.05	34044.07	0.28
	5"-COOH	**39**	$C_{21}H_{28}O_4$	-	14.56	343.19	1065349.00	100.00
				-	15.72	343.19	481593.59	19.80
		40	$C_{11}H_{14}O_3$	-	14.56	193.09	75317.45	7.07
	9'-COOH	**41**	$C_{21}H_{28}O_4$	+	15.85	345.21	150768.06	3.01
				+	20.67	345.2	21276.88	5.92
		42	$C_{13}H_{14}O_3$	+	15.85	217.09	182060.16	3.63
	DiOH	**43**	$C_{21}H_{30}O_4$	+	20.07	347.22	628388.19	75.86
				+	15.51	347.22	36306.39	3.56

*Parent Ion data gathered from MS/MS data of CBC standard.



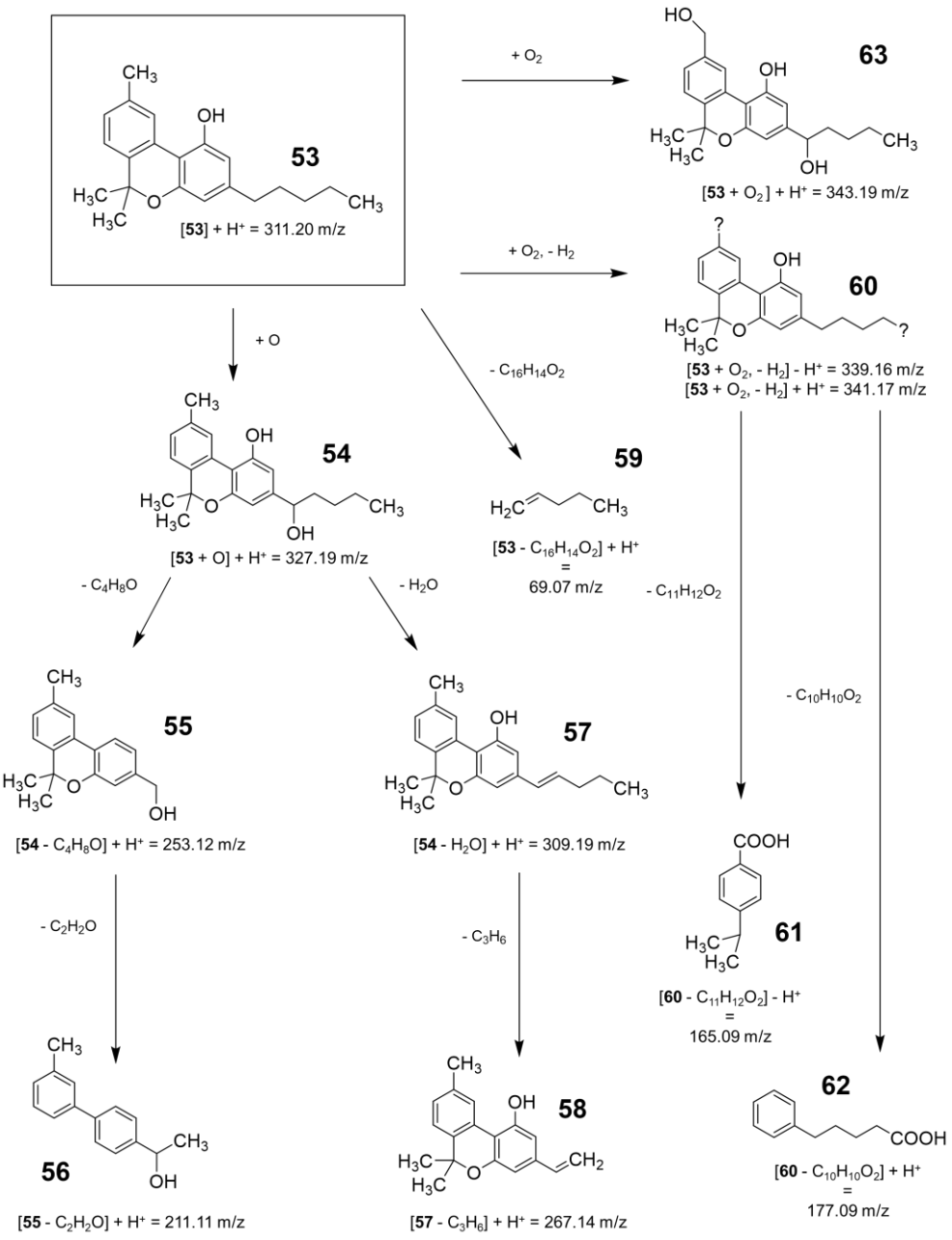
Supplemental Figure 5. Fragmentation pathway of CBD. Parent species as Ion **44**; monohydroxylated species represented as Ions **45-49**; **c**arboxylated species as Ions **50** and **51**; dihydroxylated species as Ion **52**. Corresponding MS/MS data and retention times provided in **Supplemental Table 5**.

Supplemental Table 5. MS/MS data corresponding to ions distinguishing CYP2J2-CBD metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Cannabidiol	**Parent***	**44**	$C_{21}H_{30}O_2$	+	21.24	315.23	236230496.00	100.00
	1"-OH	**45**	$C_{21}H_{30}O_3$	+	17.97	331.22	22309.22	5.28
		46	$C_{18}H_{22}O_2$	+	17.97	271.17	37226.14	8.82
		47	$C_{12}H_{18}O_3$	+	17.97	209.12	13521.62	3.20
		48	$C_{12}H_{16}O_2$	+	17.97	191.11	60162.30	14.25
		49	$C_{17}H_{22}O_3$	+	17.97	275.16	6640.29	1.57
	7-COOH	**50**	$C_{21}H_{28}O_4$	+	18.01	345.2	92596.23	21.40
				+	18.81	345.21	87302.59	20.17
				+	20.31	345.2	97335.08	65.87
				-	20.44	343.19	1490278.00	100.00
		51	$C_{15}H_{18}O_4$	+	18.81	263.13	39798.00	9.20
	DiOH**	**52**	$C_{21}H_{30}O_4$	+	11.60	347.22	9769595.00	49.97

*Parent Ion data gathered from MS/MS data of CBD standard.

**CBD-DiOH data gathered from MS data, as MS/MS spectra did not provide any ions with a mass corresponding to the unfragmented dehydroxylated fragment.



Supplemental Figure 6. Fragmentation pathway of CBN. Parent species as Ion **53**; monohydroxylated species represented as Ions **54-59**; parent 11/5”-carboxylated species is represented as Ion **60**, with question marks corresponding to either type of carboxylation, depending on the ionization mode used; 11-carboxylation (negative ion mode) represented as Ion **61** and 5’-carboxylation (positive ion mode) represented as Ion **62**; dihydroxylated species as Ion **63**. Corresponding MS/MS data and retention times provided in **Supplemental Table 6**.

Supplemental Table 6. MS/MS data corresponding to ions distinguishing CYP2J2-CBN metabolism.

pCB	Modification	Ion #	Formula	Mode	Retention Time	m/z	Intensity	Rel. Abundance in MS/MS spectrum (%)
Cannabinol	**Parent***	**53**	$C_{21}H_{26}O_2$	+	23.04	311.20	72421216.00	100.00
	1'-OH	**54**	$C_{21}H_{26}O_3$	+	19.31	327.19	7484971.00	4.42
		55	$C_{17}H_{18}O_2$	+	19.31	253.12	169165280.00	100.00
		56	$C_{15}H_{16}O$	+	19.31	211.11	6758462.50	4.00
		57	$C_{21}H_{24}O_2$	+	19.31	309.19	75666080.00	44.73
		58	$C_{18}H_{19}O_2$	+	19.31	267.14	9962170.00	5.89
		59	C_5H_{10}	+	19.31	69.07	2100366.75	1.24
	11-COOH	**60**	$C_{21}H_{24}O_4$	-	17.38	339.16	58063432.00	100.00
	11-COOH	**61**	$C_{10}H_{12}O_2$	-	17.38	165.09	321084.72	6.01
	5"-COOH	**60**	$C_{21}H_{24}O_4$	+	18.86	341.17	50217104.00	100.00
	5"-COOH	**62**	$C_{11}H_{14}O_2$	+	18.86	177.09	74082.51	0.15
	DiOH	**63**	$C_{21}H_{26}O_4$	+	15.34	343.19	19536.93	0.53

*Parent Ion data gathered from MS/MS data of CBN standard.

Ions **54-**59** suggestive of CBN-1'-OH, though may also represent CBN-11-OH, for reasons stated in the main text.